Feeding management and appraisal of recirculating aquaculture system for spiny lobster *Panulirus* spp. weaning

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Abstract. The study aimed to assess lobster fry adaptation to a formulated diet over 1-3 days while evaluating the recirculating system's performance. 1,500 pueruli lobsters (average weight: 0.31 ± 0.01 g) were placed in 30 round 80 L tanks for 30 days. Lobsters underwent various feeding strategies: formulated diet only (FD), adjusting 10% fresh diet per day (D1), every 2 days (D2), every 3 days (D3), and fresh diet only (Fr). Each treatment had five replicates. Results showed feeding strategies influenced lobster growth, with survival rates between 23%-29%. The recommended strategy was adjusting 10% fresh diet every three days, yielding the best performance in final weight (0.93 g), weight gain (187%), and specific growth rate (3.5% per day). Initial weeks saw high mortality and moulting during the puerulus to post-puerulus transition. Water quality analysis indicated the phytobiological filter had lower total ammonia nitrogen (TAN), nitrite-N, nitrate-N, and higher pH compared to other filters in use.

1 Introduction

Lobster is considered a luxurious seafood product worldwide, and the demand for lobsters has been steadily increasing, particularly in East Asian countries, driven by growing prosperity [1]. This surge in demand, especially from China, has created economic opportunities for coastal communities in tropical areas. Consequently, spiny lobsters (Palinuridae) have emerged as economically significant marine crustaceans for Indonesia and Vietnam, which are both rich in natural lobster fry resources. The wild lobster fries are typically captured in the delicate and transparent puerulus stage, as this stage allows for convenient transportation in large quantities over long distances. Before being placed in lobster grow-out cages, the pueruli undergo a nursing process to reach a juvenile size [2]. However, this early weaning stage poses the highest vulnerability in lobster farming.

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In the early nursery phase, there is a shift from natural feeds to farm-prepared fresh feeds, which include fish, mollusks, and crustaceans. Fresh feeds release chemoattractants that are favored by the fry, and their soft texture is preferred by the less developed mouthparts of the fry [3]. However, the use of fresh feed often results in lower survival rates, possibly due to the lack of certain nutritional components [4]. Additionally, the fresh feed has limitations, including short shelf life, the need for freezing to maintain freshness, inconvenience in large-scale preparation, adding organic burden in the waters, and the risk of introducing pathogens to the lobster fry [5].

Artificial feed has been introduced as an alternative to overcome the limitations of fresh feed. However, artificial feed also has its shortcomings. It has a dry texture that is less preferred by lobster fry and less attractive to their chemical sensors [3]. Moreover, artificial feed generally contains lower proportions of protein and lipid compared to fresh feed (on a dry matter basis) and can lead to nutrient leaching issues [6]. These limitations can be addressed by formulating the artificial feed in a moist form, incorporating fresh feed ingredients, and adding essential fatty acid sources, including feed attractants, pigments, probiotics, and immunostimulants [7]. Aside from the physical and chemical aspects of the artificial feed, the adaptation process itself can influence the acceptance of the feed by lobsters. This research aims to investigate the adaptation of formulated feed for lobster fry through a gradual transition to artificial feed and assess its effects on growth and survival.

Typically, many lobster fries are weaned in cages, and in this experiment, the lobster fries are reared in a recirculating system. To enhance the performance of the filtration system, a multi-loop system is implemented. In this system, the water flow within the filtration system is separated from the water flow toward the lobster-rearing tank [8]. This design ensures that the presence of the filter does not impede the main water flow. In case any issues arise with the filter, the components can be quickly repaired or replaced without significantly impacting water quality. This research aims to evaluate the differences in water quality at the output of each filter in the filtration system.

2 Materials and Methods

2.1 Pueruli handling

A total of 4,000 transparent pueruli of unidentified species were collected by fishermen from Pulo Raya, Sampoiniet, Aceh Jaya, Aceh, Indonesia (latitude 4.87, longitude 95.40). These pueruli were then transported to the Brackishwater Aquaculture Development Center Ujung Batee laboratory (latitude 5.65, longitude 95.42, Aceh, Indonesia). Inside a square fiberglass tank measuring 50x160x200 cm, the pueruli were stocked in a soft-textured hapa, which provided shelters made from butterfly-mode tied hapa and cut pipes. The pueruli were supplied with aerated and recirculated seawater at a rate of 50 L/m, a temperature range of 26-30°C, pH 8.1-8.8, and a salinity of 28-30 g/L.

Initially, the lobsters were fed a mixture of oyster, crab, and trash fish obtained from a local supplier in Banda Aceh. They were given the feed ad libitum for adaptation purposes and to anticipate metamorphosis to post-puerulus stages. After two days, the lobsters were weighed, and a group of 1,500 lobsters with similar weight and good health were selected for the experiment. The remaining lobsters were transferred to an outdoor concrete tank. At the end of the experiment, the early juvenile lobsters were identified, and the proportion was calculated based on the remaining population.

2.2 Diet preparation

The fresh feed used in the system included a combination of oysters (*Crassostrea* sp.), crabs (*Portunus* sp.), and trash fish (*Rastrelliger* sp.), which were obtained fresh from a local market in Banda Aceh. The fresh ingredients were collected, weighed, and placed in plastic bags according to the required amounts, and then stored in a freezer. They were transferred to a temperature of 10°C one day before feeding.

The formulated feed utilized in the system was provided by the Gondol Mariculture Institute in Bali and was formulated under the supervision of Dr. Clive M. Jones (College of Sciences and Engineering, James Cook University, Australia). The composition of the formulated feed is as follows: imported fish meal (65.3%), fresh fish (6%), fresh mussels (6%), fresh squid (1%), wheat flour (6%), wheat gluten (6%), mannan oligosaccharides (0.5%), fish oil (2.6%), astaxanthin (1%), cholesterol (0.5%), lecithin (1.7%), mineral and vitamin premix (1.7%,): a kilogram contained manganese 400 mg, selenium 20 mg, iron 6,000 mg, zinc 2,200 mg, copper 700 mg, iodine 6 mg, vitamin A 200,000 IU, biotin 20,000 mcg, vitamin E 6,000 IU, inositol 6,000 mg, nicotinic acid 3,929 mg, vitamin B12 2,000 mcg, vitamin B6 1,000 mg, vitamin B2 800 mg, vitamin D3 100,000 IU, choline chloride 8,000 mg, panthotenic acid 1,500 mg, vitamin B1 1,000 mg, folic acid 200 mg, vitamin K3 100 mg), vitamin C (Stay-C^{TM}) (0.4%), and binder (carboxymethyl cellulose) (1.3%). The proximate analysis of the feed is crude protein 52%, crude lipid 18.5%, and ash 15.5%. The formulated diet was weighed, stored in plastic bags, and kept at a temperature of 10°C.

2.3 Experimental design

The study was designed using a completely randomized design with five different dietary treatments for lobsters: a formulated diet only (FD), 10% fresh diet replacement per day (D1), 10% fresh diet replacement every 2 days (D2), fresh diet replacement every 3 days (D3), and a fresh diet consisting of fish, crab, and mollusk (Fr). Each treatment was replicated in five tanks. A group of lobsters with uniform size (mean ± SD, 0.31 ± 0.01 g) was randomly selected, weighed, and placed into 25 round plastic containers in a recirculating system, with 60 lobsters per tank. The weight of the puerulus was measured individually at the beginning of the experiment and then weekly.

The feeding ratio for the fresh diet was set at 30% of the body weight, while for the formulated diet, it was 7.5% of the body weight. The feeding ratio for the second to fourth treatments was adjusted based on the change in feed type according to the specified time. For example, in the first treatment (10% fresh diet replacement per day), the amount of feed for the first day was calculated as follows:

$$\textit{Total feed amount} = \textit{Formulated feed} + \textit{fresh feed} \quad (1)$$

$$\textit{Formulated feed} = \textit{Total body weight} \times 7.5\% \times 90\% \quad (2)$$

$$\textit{Fresh feed} = \textit{Total body weight} \times 30\% \times 10\% \quad (3)$$

On the subsequent day, the formulated feed was reduced by an additional 10%, while fresh feed was increased by 10%.

The feeding schedule consisted of two feedings per day, at 9 a.m. and 4 p.m. in Western Indonesia Time. Any uneaten feed and feces were siphoned out daily in the morning, before feeding.

2.4 Survival and growth observations

The number of dead and moulted lobsters was recorded daily, and the data was subsequently presented in a graph. The feeding response of the lobsters was also observed throughout the experiment. Additionally, the lobsters were weighed at the beginning of the experiment, every week, and the final day. The calculations for survival and weight measurements were conducted as follows:

Survival rate/SR (%) = 100 x (*final number of fish/initial number of fish*) (4)
Weight gain/WG = 100 x (*final weight – initial weight*)/*initial weight* (5)
Specific growth rate/SGR (%) = 100 x (log_e *final weight* - log_e *initial weight*)/*number of days* (6)

2.5 Multi-loop recirculating system

The recirculating system is situated within a 10x10 m^2 building that features transparent polycarbonate roofs and walls. This design allows natural sunlight to illuminate the system for 12 hours of light and 12 hours of darkness. Seawater is extracted from the sea and enters the sedimentation tank before passing through a sand filter. It is then transferred to the reservoir of the recirculation system using a filter bag.

The recirculating system comprises lobster-rearing holding tanks, a reservoir, and filters. The rearing tanks are round, black plastic tanks with a capacity of 100 L (56 cm in diameter, 46 cm deep), with an effective volume of 80 L. Each tank is equipped with a line of aeration through a transparent PVC hose, and a small shelter made from tied hapa is provided. The tanks are covered with black hapa for shading. The water flow is facilitated by a water pump with a flow capacity of 50 L/min (Amara AA-105, China). Water is delivered from the reservoir through a 1-inch pipe and distributed to each tank via a ¾-inch pipe connected to a tap. The water flows out of the tank through the top side of a 1/2-inch outlet pipe into a 2-inch outflow pipe.

The effluent from the system, known as the "E," is directed back to the reservoir. The reservoir is divided into three chambers using two layers of green screen. The first chamber houses two detritivorous fish, approximately 50 g each, such as seawater-adapted tilapia (*Oreochromis niloticus*) and green mullet (*Mugil cephalus*). These fish help consume settling organic wastes. The water then flows through the green screen layers and mixes with the filtrate from the second and third pool subsystems. Finally, the water is pumped back to the rearing tanks, completing the main loop of the system. For a visual representation, please refer to Figure 1.

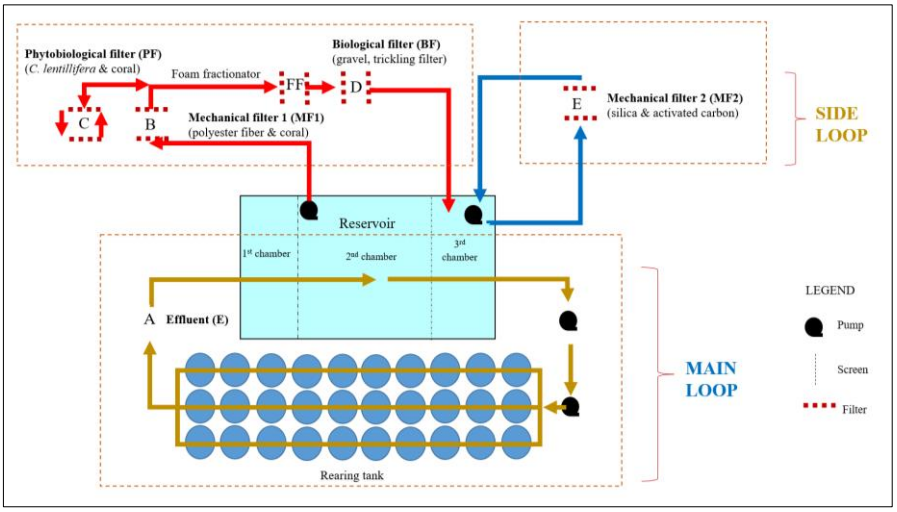

Fig. 1. Arrangement of components of the multi-loop recirculation system for lobster weaning (image not to scales)

The water from the second chamber is delivered by a pump (same type as above) to the filtering system, creating the second loop of the subsystem. The water flows through an aerated pipe containing an airstone, then into a 2" perforated pipe wrapped with green screen

before falling into the first mechanical filter (MF1), a 100 L conical tank (100 cm diameter, 130 cm deep) filled with 65x52 cm polyester fibers (Dacron^{TM}) and coral stone (22 kg, volume 13.32 L, 132 pieces). There is a coupled side of interconnected conical tank (100 L,) that functions as a phytobiological filter (PF) filled with 150 g of *Caulerpa lentillifera* and coral stones. The parallel position of both tanks is designed to reduce remaining organic particles from previous filtration that may cover the seaweed, which can impede its work. An airlift system is used to move water from the bottom of the tank to the seaweed platform. The filtrate then flows into a foam fractionator (10 cm diameter, 120 cm height) before being directed to two sequential tanks (each 50x30x30 cm) of a biological filter (BF) containing gravel stone and coral stone. Please refer to Figure 2 for visualization.

In the third chamber, the water is filtered by the second mechanical filter (MF2): two tube filters (Nannotec FRP 1354, USA) containing silica sand and activated carbon (3/4 of its volume), creating the third loop of the subsystem. An independent pump (Amara AA-105, China) is used to transfer the water into the filter and back to the reservoir.

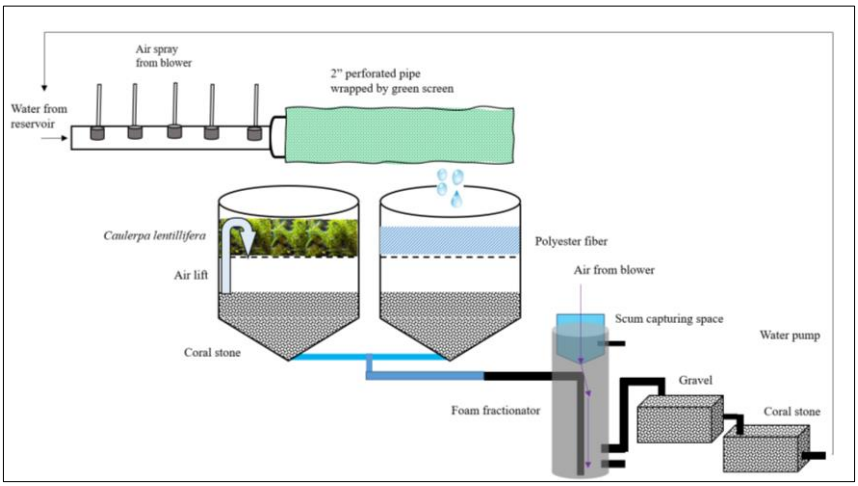

Fig. 2. Second loop of filtration system (image not to scales)

2.6 Measurements in recirculating system

The systems were operated for two weeks before starting the experiment (to develop nitrification bacteria), during which two saline tilapia and green mullet were reared in a reservoir. At the beginning of the experiment, 150 g of *Caulerpa lentillifera* was added as a component. The growth of seaweed biomass was assessed every week using a specific formula (Formula 5). The growth of the seaweed was supported by natural sunlight.

Water quality parameters were monitored to ensure the stability of the system. Dissolved oxygen (DO), pH, salinity, and temperature were measured twice a week, both in the morning (9 a.m.) and in the afternoon (4 p.m.). Furthermore, total ammonia nitrogen, nitrite, and nitrate levels were measured twice a week, specifically at 9 a.m. These measurements were conducted on both the effluent and water samples obtained from different filters within the system.

E = Effluent, water that comes directly from the main pipeline of the rearing tank outlet

MF1 = First mechanical filter, the filtrate from the conical tank that contains polyester fibers and coral stone

PF = Phytobiological filter, the filtrate from the conical tank that contains seaweed and coral stone
BF = Biological filter, the filtrate from the conical tank that contains gravel and coral stone
MF2 = Second mechanical filter, the filtrate from tube filter that contains silica sand and activated carbon

TAN (Total Ammonia Nitrogen) was measured using a spectrophotometer (U-1500, Hitachi) with the phenate procedure. A 25 mL sample of water was placed in a 50 mL volume tube, followed by the addition of 1 mL of phenol solution and homogenization. Then, 2.5 mL of the oxidizing solution was added and homogenized again. The tube was covered and left undisturbed for 1 hour for color formation. The solution was transferred to a cuvette in the spectrophotometer, and the absorbance was read and recorded at a wavelength of 640 nm. Nitrite-N was measured using a colorimeter (colorimeter DR/890 HACH). A 10 mL sample of water was placed in a tube and mixed with NitriVer 3 reagent. The solution was homogenized and left for 15 minutes. Then, it was measured using the DR/890 Colorimeter at a wavelength of 420 nm. Nitrate-N measurement was conducted using the same colorimeter as for nitrite measurement. A 10 mL sample of water was placed in a tube and mixed with NitraVer 5 Nitrate reagent, then left for 5 minutes. It was then measured using the DR/890 Colorimeter at a wavelength of 420 nm. A YSI 550A DO meter was used to measure dissolved oxygen, and a pH meter (Hanna HI98128) was used to measure pH and temperature. Salinity was measured using an ATC handheld refractometer.

2.7 Data analysis

The data on survival, weight gain, and specific growth rate were analyzed using a one-way ANOVA (analysis of variance) with a significance level of 95%. The assumption made was that the variance is homogeneous. If necessary, a Duncan post hoc test will be conducted for further analysis. The software used for this analysis was SPSS version 26.0. Graphs were used to present other data, such as moulting, survival rate, water quality, and seaweed growth.

3 Results and Discussion

3.1 Feeding responses and daily feeding quantities

The lobster showed good acceptance of the formulated diet even during the first feeding (based on subjective observation presented in Figure 3). The strong smell and bright color may have attracted them. Lobsters were able to grasp the feed easily due to its smooth texture and suitable particle size. In a gram of feed, there are approximately 500 feed particles. At the same feeding level (30% and 7.5% of body weight for fresh feed and dry feed, respectively), the formulated diet had a higher particle count compared to the fresh feed. A lobster fry weighing 0.3 g, with a feeding rate of 30% and two feeding times, can consume 22 feed particles.

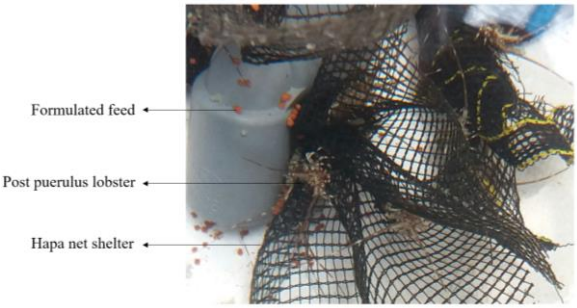

Fig. 3. A positive response of lobster fries to the formulated diet

Utilizing fresh feed derived from trash fish is a more viable option for lobster feed compared to crustaceans or mollusks. However, in the long term, it can hinder the progress of the aquaculture industry. In terms of quantity, the availability of trash fish fluctuates seasonally, posing challenges in effectively adjusting feed rates and frequency. Moreover, trash fish is prone to deterioration and has variable and suboptimal nutritional profiles, resulting in poor food conversion ratios and negative environmental impacts [4].

In contrast, dry feeds present a range of benefits, such as convenient and affordable storage, conversion into marketable products, adaptability in feeding methods, and crucially, enhanced environmental sustainability [4]. However, in the perceptions of lobster's farmers may not readily accept dry feeds [9]. They require a transitional period to adjust to moist pellets or a combination of fresh ingredients with dry feed options. At least 15% of moisture has to be in the diet [7]. In our findings, we discovered that the perceptions of farmers are not always accurate. We found that the lobster fries can consume dry feed and respond to it actively (Figure 3). The response of the fries decreased when the feed was soaked for a certain period in water.

However, increasing the feeding frequency may help resolve this issue. *Panulirus versicolor* exhibited improved carapace length when fed four times, suggesting a recommendation for more night feeding due to its nocturnal behavior [10]. On the other hand, *Panulirus ornatus* showed increasing growth from one feeding session to sixteen feeding sessions throughout the day [11]. An unresolved issue in our study is the unidentified species of lobster fries, which hinders the analysis of each species' behavior. This challenge arises from the difficulty of identifying species during the transparent phase of lobster development.

3.2 Growth and survival

The study revealed significant differences in growth performances ($p<0.05$) among the treatments (Table 1). The weight gain and specific growth rate of lobsters in D3 (fresh diet replacement every 3 days) were higher compared to those without replacement (fed formulated diet (FD) or fresh diet (Fr)). Within a month, the weight of post-pueruli in the D3 treatment nearly tripled from the first day. After a month of weaning, the average weight of pueruli increased from 0.32 g to 0.93 g. The other replacements, D1 (every day) and D2 (every 2 days) did not show significant differences among themselves or compared to other treatments ($p>0.05$).

Table 1. Growth and survival of lobster fries treated by different times of gradual replacement of fresh diet to formulated feed

Treatment	Initial weight (g)	Final weight (g)	Weight gain (%)	Specific growth rate (%)	Survival (%)
FD	0.31±0.01	0.76±0.02	141±3 ^{a}	2.94±0.04 ^{a}	26.6±1.9^{a}
D1	0.31±0.01	0.79±0.02	154±9 ^{ab}	3.09±0.12 ^{ab}	23.0±1.4 ^{a}

D2	0.31±0.01	0.79±0.04	157±2 ^{ab}	3.12±0.20 ^{ab}	28.7±0.8 ^{a}
D3	0.32±0.01	0.93±0.02	187±9 ^{b}	3.50±0.10 ^{b}	28.0±2.1 ^{a}
Fr	0.32±0.00	0.71±0.03	124±1 ^{a}	2.67±0.18 ^{a}	28.0±1.4 ^{a}

The results are presented as means ± standard error. Data with different superscripts within the same row indicate significant differences p<0,05, n_0 = 60

The survival of lobsters was not significantly different among the treatments ($p>0.05$) (Table 1). At the end of the experiment, the average survival rate ranged from 23% to 28.7%. Based on the average data, the highest mortality occurred during the pueruli stage (50-60%). Upon closer examination, the fresh diet treatment experienced higher mortality compared to other treatments, while the formulated diet treatment showed more persistence (Figure 4a). In the following week, the lobster fries undergoing daily adaptation with the formulated feed had the lowest survival rate.

During the first week, the pueruli metamorphosed into the post-pueruli stage. It was observed that the most frequent occurrence of molting in the fries (approximately 17%) was observed in all treatments (Figure 4b).

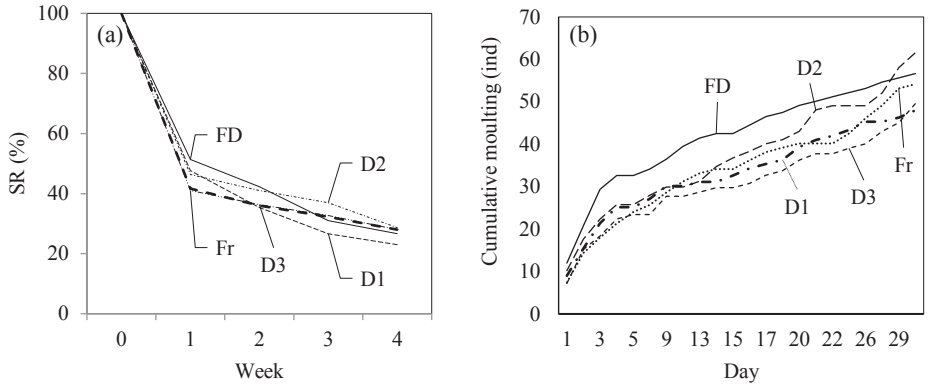

Fig. 4. Lobster decreases survival mostly in the pueruli stage (a), lobster population moulting gradually. The first week showed the most number of moulting fries (b). The graphs were presented based on means of treatment (n_0 = 60).

At the end of the experiment, the total population of fries had metamorphosed to the post-larval stage. It was observed that more than half of the remaining population consisted of *Panulirus ornatus* (57%). Following closely, *Panulirus homarus* (31%) made up approximately one-third of the population. The remaining population was comprised of *Panulirus longipes* (6%), *Panulirus versicolor* (4%), and *Panulirus penicillatus* (1%) (Figure 5).

Panulirus ornatus | *Panulirus homarus* | *Panulirus longipes* | *Panulirus versicolor* | *Panulirus penicillatus*

Fig. 5. Species of *Panulirus* spp. in the remaining lobster population.

The findings suggest that a combination of a formulated diet and a fresh diet enhanced growth performance (Table 1). Previous studies by Irvin and Shanks (2015) have indicated

that during the early juvenile stage, the inclusion of fresh ingredients and a high moisture content has a more significant impact than incorporating high protein levels [4]. Similarly, Smith et al. (2005) demonstrated that feeding *Panulirus ornatus* with mussels alone resulted in lower growth performance compared to using formulated feed [12]. In line with these findings, the feed used in this study contained a protein content deemed nearly ideal that is 52% as suggested more than 50% by William, 2007 [13], and it also included an adequate amount of fresh ingredients, accounting for 13% of the total weight of the feed.

Incorporating fresh ingredients into the diet aligns with the natural feeding behavior of lobsters, which involves consuming a variety of invertebrates such as molluscs, crustaceans, polychaete worms, and echinoderms. This dietary preference characterizes spiny lobsters as opportunistic carnivores that have evolved to efficiently utilize food sources that are high in protein, low in lipid content, and contain moderate to high amounts of carbohydrates. This is because glycogen, which is the primary energy store in molluscs, typically accounts for 14-24% of the ash-free dry matter [13].

The fresh diet offers the advantage of higher protein content based on dry matter, but it is less energy-efficient due to its high moisture. To address this drawback, formulated diets are developed to provide a solution. Formulated diets allow for the inclusion of functional ingredients that support the health of lobsters. In this research, examples of such ingredients include astaxanthin, cholesterol, and MOS (mannan oligosaccharides). Studies have shown that in adult *P. ornatus*, the highest concentration of astaxanthin and lower concentration of cholesterol result in optimal growth [11]. While these ingredients do not directly affect the growth and survival of *P. ornatus* in the juvenile stage, their accumulation in the lobster's body can enhance immunocompetence and potentially increase market price [12]. Additionally, MOS has been proven effective in improving the growth, survival, and gastrointestinal health of *P. homarus* lobsters [14].

However, this study reveals that the combination of these ingredients alone is not sufficient. The formulated diet should be gradually introduced in smaller proportions and over a longer period. The most satisfactory approach is to replace 10% of the fresh diet every three days. This gradual adaptation process takes approximately one month to achieve the best growth of lobsters. Goliath grouper (*Epinephelus quinquefasciatus*) requires an even longer period of adaptation, lasting ten weeks, with a gradual change of 20% every two weeks [15]. Another practice, known as co-feeding, which involves a mixture of live and pellet feed, is applied to pikeperch (*Sander lucioperca*) [16]. In this method, the live feed is initially mixed with pellet feed for a period of 10-12 days. Over time, the proportion of live feed is reduced, while the proportion of pellet feed is increased by 25% every two days until the fish are solely consuming pellet feed. This rearing method has been successfully implemented in several European countries, resulting in the stable production of high-quality pikeperch juveniles [16,17]. Similar practices have also been applied to walleye (*Sander vitreum*) for a duration of 10-21 days [18].

The survival of lobsters during the pueruli stage exhibits a sharp decline (50-60%) in the first week (Figure 4a.). During this stage, the pueruli are transparent, and their color only becomes visible after molting when they enter the post-pueruli stage. It was previously believed that the puerulus stage is a non-feeding stage, relying on energy reserves from the previous stage known as phyllosoma [19,20]. However, recent findings using eDNA metabarcoding techniques have detected the DNA of zooplanktonic crustaceans in the stomach content of pueruli, questioning the previous hypothesis [21]. Drawing upon these findings, we suggest that the decreased survival rate observed during the transition period from the puerulus to the post-puerulus stage may be attributed to inadequate nutrient storage, especially lipid, in terms of quantity and quality. This hypothesis aligns with the review of Francis et al. (2014) [22].

Based on the average values, it is evident that the formulated diet had a positive impact on the moulting process of the pueruli, as indicated in Figure 4b. Notably, there is a noticeable difference in the number of moults between the formulated diet and the fresh diet, with the largest gap observed in the first week. Throughout the experiment, the gap remains consistent, particularly in the D3 treatment. Another interesting finding is that the increased frequency of moulting in lobsters does not necessarily correspond to higher growth rates. This finding aligns with the observations of *P. versicolor*, which showed a non-linear relationship between moulting and growth when fed a formulated diet [10].

3.3 Water quality

All the fundamental water quality criteria for *Panulirus* spp. remained within acceptable ranges. In Figure 6a, the fluctuation of total ammonia nitrogen (TAN) during the first week is depicted. The phytobiological filter (PF) had the lowest value (<0.2 mg/L), while the others ranged between 0.9 and 1.3 mg/L of TAN. Over the course of the week, TAN levels stabilized at less than 0.1 mg/L until the end of the experiment.

Figure 6b demonstrates that nitrite-N consistently remained below 0.3 mg/L throughout the experiment. PF had the lowest value (<0.2 mg/L) at various points, followed by mechanical filter 2 (MF2). Mechanical filter 1 (MF1) exhibited the highest value at certain times and never reached zero, except on the initial day. Other filters recorded the lowest nitrite concentrations. In Figure 6c, it can be observed that nitrate-N remained below 50 mg/L throughout the experiment. The values of most filters were relatively similar, with divergence occurring in the second week when nitrate levels significantly increased. E and BF came together in opposition to PF and both MF filters. In other instances, the values were mostly below 25 mg/L.

The temperature primarily remained above 28°C. There were only slight deviations below this threshold at the beginning and end of the experiment, with a difference of half a degree or one degree lower. However, PF exhibited higher temperatures than the other filters for several consecutive days, specifically from the 11th to the 22nd day. For more details, refer to Figure 6d.

Dissolved oxygen formed distinct clusters. The first cluster consisted of effluent (E) and end of filtration (MF2), while the second cluster comprised MF1, PF, and BF. These clusters were separated by an oxygen concentration of 6.5 mg/L. However, the first cluster also displayed a clear separation in the second week, indicating that E had higher values than MF2.

The pH data consistently remained above 8. It decreased only during the first week and gradually increased in the second week, stabilizing thereafter. Upon closer examination, PF exhibited higher values compared to the others, while the lowest values were generally found in MF2.

Salinity was not presented in the graphs as there were no significant variations observed across the treatments. The salinity level ranged from 26 to 30 ppt (parts per thousand) in all treatments.

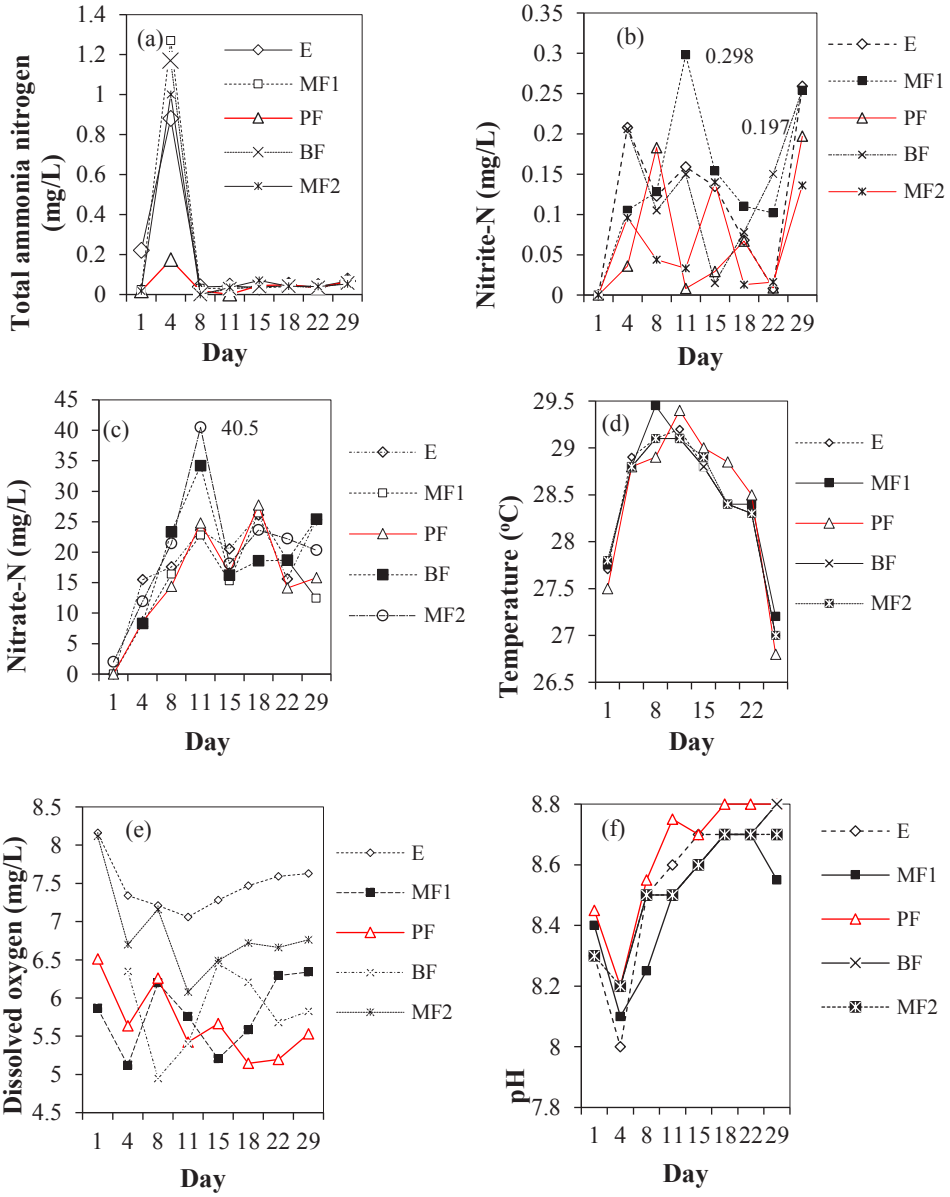

Fig. 6. The graphs show several water quality parameters measured in a recirculating system based on the output source of the filter, namely total ammonia nitrogen (a), nitrite-N (b), nitrate-N (c), temperature (d), dissolved oxygen (e), and pH (g).

Goddek (2017) implemented a decoupled system by separating the recirculated aquaculture system (RAS) and hydroponic (HP) units, aiming to capitalize on the advantages of both systems. This approach was chosen due to the different response times of fish growth and plant development, with the former occurring over hours or days as a slow process, while the latter involves photosynthesis and transpiration happening within seconds or minutes as fast processes [8]. In our design, special attention was given to ensure that the presence of the filter in the RAS does not hinder the main water flow. Additionally, the system was

designed to facilitate quick repairs or replacements of filter components, minimizing any potential negative impacts on water quality.

In assessing the efficiency of filtration systems, inorganic nitrogen compounds are commonly used as criteria [23]. However, it is important to note that the purpose here is not to directly compare the filters but rather to examine the sequential results of filtration. For instance, the main effluent exhibits high levels of dissolved oxygen due to the relatively low biomass of lobsters and feed (initially 225 g/m^3 with a feed load of 16.87 g/m^3/day). Conversely, the dissolved oxygen level is lower in the first mechanical filter, which consists of polyester fiber. This is typically caused by the accumulation of organic matter, leading to localized reduction of oxygen in the water. The presence of organic matter stimulates the growth of heterotrophic bacteria and inhibits the growth of nitrifiers [24]. Additionally, the organic matter accumulation may promote the conversion of nitrate to nitrite through aerobic nitrate respiration (ANR) [25].

After one week, the growth of nitrifiers in the system appeared to be effective. On the other hand, *Caulerpa lentillifera*, as a phytobiological filter (PF), exhibits higher ammonia absorption compared to other filters, with a lower conversion into nitrite or nitrates. In the crab recirculating system, *C. lentillifera* showed better efficiency in absorbing ammonia compared to the control group. However, it was found to be less effective in ammonia absorption compared to other species of seaweed [26]. The presence of filamentous algae in the reservoir was observed due to inadequate light control. This growth of photosynthetic organisms could have potentially influenced the concentration of inorganic nitrogenous substances in the water.

PF water consistently displayed higher temperatures compared to the other filters over several consecutive days. Unlike the direct flow from the mechanical filter to the biological filter, the PF operates in parallel with the MF1 tanks. The water inside the PF is circulated using an air lift system from the bottom of the tank to the seaweed tray. Therefore, it is reasonable to observe higher temperatures during noontime due to the stagnant conditions in the PF.

The level of dissolved oxygen can indicate the degree of respiratory activity occurring in the filter system. The first mechanical filter, biological filter, and phytobiological filter exhibited lower oxygen content compared to the effluent and second mechanical filter. This observation can be attributed to the fact that aquatic plants utilize oxygen for respiration during the night or under cloudy and rainy conditions, while the microbes in the biofilter utilize oxygen for the oxidation of ammonia to nitrites and nitrates [27]. In contrast, the pH data consistently remained above 8 throughout the duration of the experiment. Upon closer examination, it is evident that PF consistently exhibited higher pH values compared to the other treatments. This can be attributed to the presence of aquatic vegetation, which contributes to the increase in water pH as it absorbs bicarbonate for photosynthesis [28].

In the end, the elevated dissolved oxygen (DO) levels observed in the effluent, after a temporary decrease in the filtrate, indicate that the wastewater has undergone significant reduction by the end of the filtration process (MF2).

3.4 Growth of *Caulerpa lentillifera*

The growth *of Caulerpa lentillifera* experiences a significant decline, with a decrease from 57% to 25%, observed after two weeks. This decline coincides with a reduction in the daily feed amount due to the occurrence of mortality, leading to a decline in ammonia levels. This significant decrease in nutrient input ultimately halts the growth of the seaweed. See Figure 7.

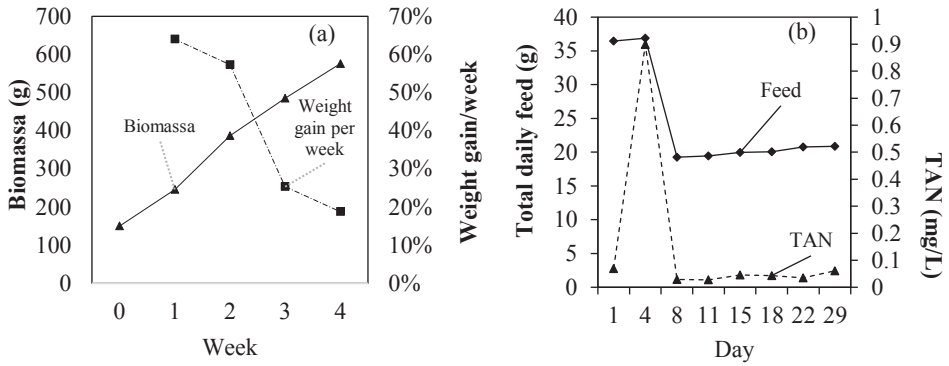

Fig. 7. The growth of the seaweed *Caulerpa lentillifera* exhibits a rapid decline after one week (a), reducing the total daily feed amount could result in a significant decrease in TAN concentration during the second week (b).

In terms of climate change resilience, the recirculating aquaculture system operates within a controlled indoor environment, which minimizes its susceptibility to various climatic factors. By reducing exposure to these climate-related risks, the system enhances the stability and reliability of aquaculture production. Nonetheless, energy consumption and greenhouse gas emissions pose significant challenges and constraints to the widespread adoption of it [29].

Many scientists acknowledge the role of seaweed in reducing greenhouse gas emissions and bolstering the biocircular economy [30-34]. This study showcased the successful utilization of seaweed *Caulerpa lentillifera* for the efficient removal of nutrients, specifically inorganic nitrogen compounds. Consequently, the seaweed will also absorb phosphate and carbon dioxide, promoting its biomass growth [35]. Seaweed holds immense potential as it constitutes a significant portion of marine aquaculture products globally (around 50%) [34].

The growth of *C. lentillifera* in this study is observed to be stimulated over two-weeks, possibly due to the release of nutrients from lobster metabolism and slow release from uneaten feed and decomposition of feces. However, previous research by Paul (2008) indicated that high concentration nutrients do not necessarily promote the growth of *C. lentillifera* significantly [36]. This discrepancy could be attributed to the insufficient bicarbonate concentration in the water, as *C. lentillifera* prefers bicarbonate over carbon dioxide for photosynthesis, as evidenced by the pH drift observed in Figure 6f [37]. *C. lentillifera* may possess CO2-concentrating mechanisms (CCM) to facilitate photosynthesis [38,39]. Additionally, the presence of coral stone in the phytobiological filter may provide bicarbonate through the reaction of carbon dioxide with coral, supporting the growth of *C. lentillifera* [40].

4 Conclusion

The study reveals that lobster fry exhibits positive responses to the formulated diet. To achieve optimal results, it is recommended to introduce the formulated diet gradually, in smaller portions, and over an extended period. The most effective approach is to replace 10% of the fresh diet every three days. This gradual adaptation process takes approximately one month and results in the best growth of lobster fry, although survival rates do not show significant differences. It is important to pay more attention to the handling and transition phase from puerulus to post puerulus stage, as higher mortality rates were observed during

this period. In the decoupled filtration system, *Caulerpa lentillifera* proves to be effective in efficiently removing nutrients, particularly inorganic nitrogen compounds.

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